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Mechanisms of interaction of acetaminophen metabolites in terms of hepatotoxicity

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ABSTRACT

The most serious complication after ingestion of a toxic dose of acetaminophen is liver damage. The aim of study was to review the research on the biochemical and molecular mechanisms of acetaminophen hepatotoxicity. It was shown that effect of the acetaminophen metabolism is the production of N-acetyl-p-benzoquinone, which is formed by bioactivation with the participation of cytochrome P450 enzymes. The combination of N-acetyl-p-benzoquinone and proteins can cause a disruption in protein homeostasis in cell membrane and mitochondrion. It may interfere with cellular signal process. Small and chronic doses of acetaminophen lead to damage of the cell nucleus. These effects can explain the mechanism of hepatotoxicity. N-acetyl-p-benzoquinone can cause an increase in oxidative stress. Lipids peroxidation and proteins oxidation are the main factors, which lead to necrosis of hepatocytes. It was shown that N-acetyl-p-benzoquinone can cause a decrease in GSH level and SH groups. For this reason, N-acetyl-p-benzoquinone was recognized as a key factor of the acetaminophen hepatotoxicity.

Keywords: acetaminophen, covalent modification of proteins, cytochrome P-450, hepatotoxicity

INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol, APAP) is a medicine from the group of nonsteroidal antipyretic and analgesics drugs used in the treatment of poor to moderate pain [2]. It is used in emergency treatment of pain, when the anti-inflammatory effect is not required. The pharmacological effect of acetaminophen is not fully understood. It is believed that the analgesic effect of the drug is dependent on the inhibition of the cyclooxygenase activity, which can induce the prostaglandin formation [2]. Acetaminophen probably suppresses the activity of cyclooxygenase-3 (COX3), a variant of COX-1 in the brain. The result of this is pain reduction and body temperature reduction to physiological values [2]. Acetaminophen can inhibit the production of mediators that activate pain receptors leading to increased levels of the pain threshold [1]. The advantage of acetaminophen is its action profile and safety of use. It can be administered both to children and pregnant women – in each trimester. According to the Food and Drug Administration classification, acetaminophen was recognized to be a category B drug. This means that acetaminophen is the drug of first choice in situations where the benefit to the mother outweighs the potential risk to the fetus, although it passes through the placenta and breast milk [12]. An important development was to demonstrate the acetaminophen applicability for people suffering from a gastric ulcer or duodenal ulcers and patients with asthma or hypertension [14].

Acetaminophen is one of the most popular over the counter (OTC) medicaments available. This promotes numerous cases of overdose. The result is an accumulation of acetaminophen metabolites in the body, which affects the liver. The aim of this study was to review the research on the biochemical and molecular mechanisms of acetaminophen hepatotoxicity.

PHARMACOKINETICS AND METABOLISM OF ACETAMINOPHEN

After oral administration, acetaminophen is absorbed by diffusion. The maximum pharmacological effect is manifested within 30-60 minutes after enteral injection. The half-life in the body is from 1.2 to 3.5 hours. The drug passes through the blood-brain barrier, does not connect

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strongly with blood proteins, and is evenly distributed in all the bodily fluids [14].

As a result of the therapeutic dose injection, the drug is metabolized in phase I of the biotransformation, where the acetaminophen structure is changed. In phase II, the drug reacts with endogenous substances, after which it is excreted from the organism in a soluble form. Following the injection of a toxic dose, the detoxifying abilities of the body are saturated and the drug is subject to an intensive biotransformation in phase I. In consequence, there is an excessive production of toxic metabolites, which connect with cellular proteins, leading to tissue necrosis.

It has been shown that acetaminophen is oxidized with the participation of cyt-P450 enzymes, mainly by CYP2E1, in phase I of the biotransformation. It leads to the formation of a reactive metabolite, namely the N-acetyl-p-benzoquinone imine (NAPQI) [3]. Young children appear to be most resistant to acetaminophen-induced hepatotoxicity because of both reduced activity of CYP2E1 and the neonate's increased ability to replete glutathione compared with adults [8]. Although, the evidence for acetaminophen caused hepatotoxicity is assigned to NAPQI, also the action of an intermediate metabolite – N-acetyl-p-benzosemiquinone imine – is considered. It has been shown that 3-hydroxyacetanilide is another important product formed during the acetaminophen biotransformation, by the action of hydroxyl radicals. In laboratory studies on animals in which the hepatotoxicity was induced by acetaminophen, metabolites such as 3-thiomethylaminophen (and its sulfate, glucuronide, sulfone derivatives), p-aminophenol and the mercapturic acid conjugates of p-benzoquinone (PBQ) were detected in urine [1].

It has been observed that following phase II of the biotransformation acetaminophen is converted into non-toxic compounds: acetaminophen glucuronide and sulphate, removed with the urine or gall. The studies of the hepatic flow (via the portal vein) indicate an increased glucuronidation ability rather than sulphation [1, 10]. NAPQI, produced in small quantities, is conjugated with GSH and then excreted with urine in the form of a mercapturic acid. However, when large reactive metabolite quantities are formed, it binds covalently with the SH groups of proteins, leading to the destruction of liver cells (Fig. 1) [10].

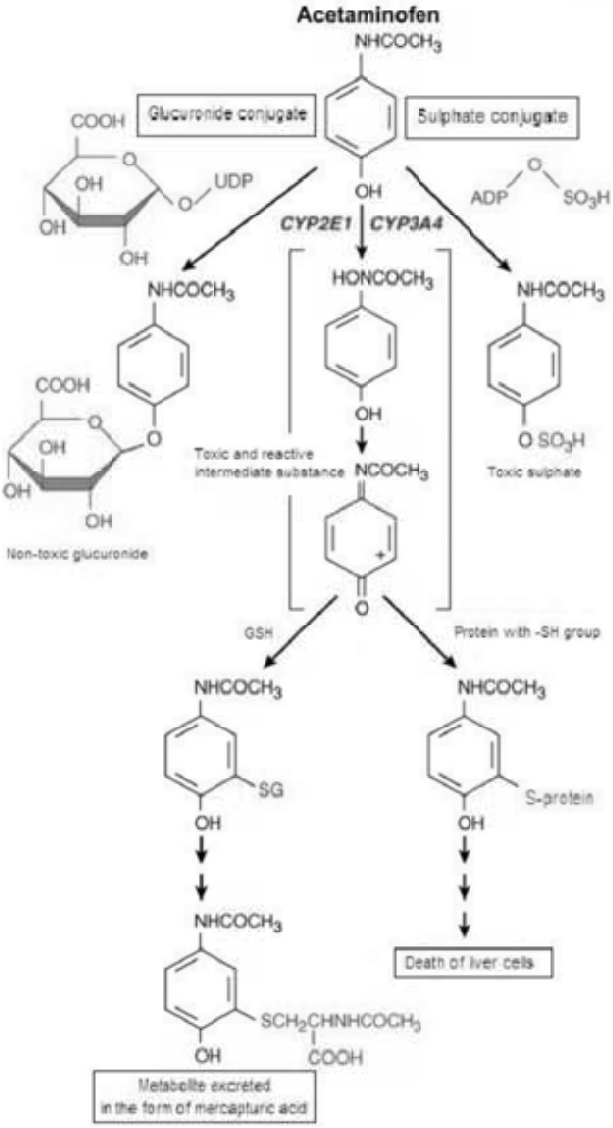
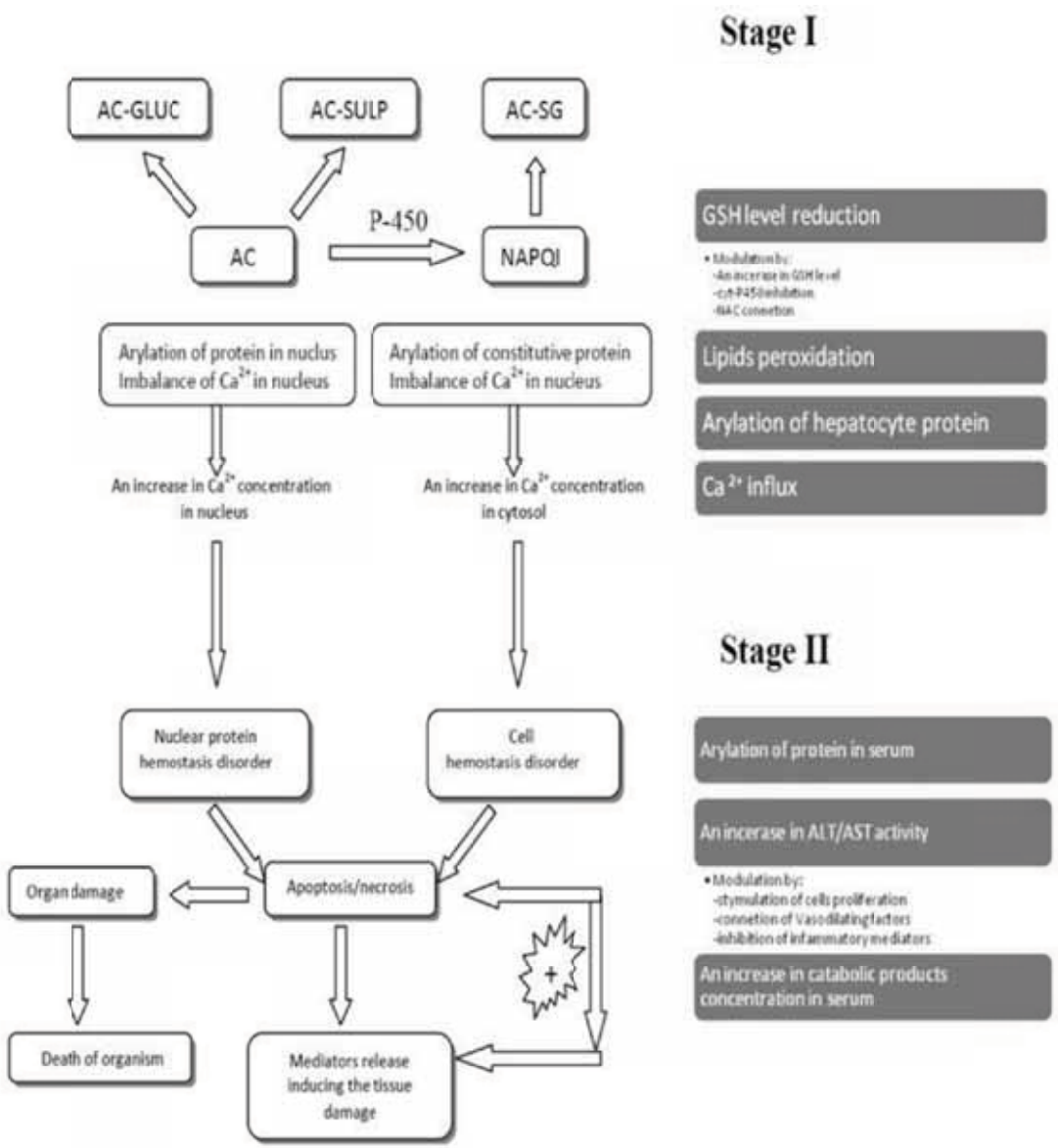


Fig. 1. Acetaminophen metabolism in the human body [10]

MECHANISM OF ACETAMINOPHEN HEPATOTOXICITY

Although the events leading to acetaminophen poisoning are known, the exact mechanism of cell destruction and disintegration is not fully understood yet. It is most probable that NAPQI binds directly with the protein molecules, causing the destruction of their structures, or that there is an indirect action – through the metabolic chain disorder – as a consequence of the processes of oxidation, lipid peroxidation and inflammation, which precede the protein adduct formation [13]. The hepatotoxicity can be divided into two stages*:* initial changes in hepatocytes and hepatocyte damage (Fig. 2) [1].

The oxidative stress is a mechanism postulated to play an important role in the early changes in the cells. It may be mediated by reactive oxygen species (ROS) or by direct oxidation of acetaminophen metabolites. It has been shown that the oxygen reduction to free radicals O_2 leads to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radicals. These reactions were catalyzed by the superoxide dismutase (SOD) and the cyt-P450 enzymes. The H_2O_2 source has not been fully identified. It probably is the reactive metabolite – NAPQI - itself. The role of oxidative stress in acetaminophen hepatotoxicity has been confirmed in studies on mice and rats. It has been shown that SOD action leads to the acetaminophen toxicity reduction [7]. Oxidative stress is also a consequence of the



AC – acetaminophen, P450 - P450 cytochrome, NAPQI – N-acetyl-p-benzoquinone imine, AC-SULP – acetaminophen sulphate, AC-GLUC – acetaminophen glucuronide, AC-SG – a combination of acetaminophen and glutathione [1].

Fig. 2. Phenomena induced hepatotoxic effects of acetaminophen – stage I and II

nitric oxide (NO) action. Reactive nitrogen species react with peroxide radicals, producing nitrogen peroxide ($ONOO^-$), which has oxidizing and nitrating properties with regard to the tyrosine residues [6]. The presence of $ONOO^-$ resulting in the protein oxidation was confirmed, however the deficiency of NO leading to the lipid peroxidation by O_2 was observed [7]. An immunohistological analysis of the liver tissue indicates that compounds arising as a result of the oxidizing effect of NO, occur in the same cells, in which the connections between acetaminophen metabolites and proteins were formed. $ONOO^-$ bonds with tyrosine leading to the necrosis of hepatocytes was observed. Studies have shown that the main oxidation mechanism at a reduced inducible nitric oxide synthase activity was the nitration of tyrosine groups. However, in the presence of an enzyme inhibitor, the main cause of hepatotoxicity was the lipid peroxidation [7].

In therapeutic acetaminophen doses, NAPQI was attached to SH groups of glutathione (GSH), so it was effectively detoxified. The high concentration of the metabolite or GSH level reduced by the oxidative stress can cause NAPQI to be released into the cell and to get attached to the membrane of hepatocytes, which results in the lipid layer damage and tissue necrosis. This reaction was catalyzed by the glutathione transferase, for which NAPQI was one of the substrates. This explains why the hepatocellular damage is not observed until decreased GSH levels. When a dose exceeding the minimum toxic amount was introduced into the body, acetaminophen etabolite reducing the GSH level to 80-90% was observed. The reduced GSH could not compensate for NAPQI and the oxidation processes increased. The GSH level decrease to 90% is considered a critical value, above which the liver cell necrosis is inevitable [6, 13]. It has been

shown that a reduction in the mitochondrial GSH level is positively correlated with the liver damage [12]. An acute liver failure (ALF) may be caused by a single overdose of acetaminophen (10-15g) or may be a consequence of a chronic treatment with low doses (g/day) [9, 15]. The degree of acetaminophen hepatotoxicity dependency on the liver condition, age over 40 and genetic predisposition, all resulting in the cyt-P450 isoenzyme polymorphism, was shown [3].

Numerous studies have shown that acetaminophen toxicity always occurs together with a covalent protein connection [1, 3, 6, 7]. NAPQI, which can oxidize cysteine amino groups, leading to the loss of protein activity or function and a possible cell lysis or death, was observed [6]. The contributing factors were the following: oxidative stress, nitrotyrozine formation, effects of inflammatory cytokines as well as changes in the mitochondrial membrane permeability [7]. Adducts of acetaminophen metabolite and proteins were an indicator of pathology appearing in intrahepatic cells, entailing a fact that their concentration may be a marker of a severe overdose.

It has been demonstrated that the acetaminophen treatment results in a decrease in the microsomal activity of Na^+/K^+ ATPase, which is probably involved in the process of organ temperature regulation. The Na^+/K^+ ATPase activity is dependent on the content of phospholipid acid, sulfhydryl groups, and phosphatidylserine in the membrane. Changes in the composition of lipids, SH groups in microsomes as well as in the lipid peroxidation lead to adverse effects in the distribution of the mitochondrial membrane microenvironment and to a reduction of the content of proteins containing SH groups. It has been shown that acetaminophen can reduce the amount of SH groups and decreases the Na^+/K^+ ATPase activity [11]. The almost total decrease of the content of SH groups may be important in fighting the harmful effects of NAPQI. Acetaminophen's toxic effect on the liver by affecting the phospholipid variation in the biosynthesis and metabolism can result in an increased membrane fluidity [11]. Acetaminophen can damage the lysosomal membrane of lipids and can cause lysosomal enzyme abnormalities, which demonstrates that toxicity can manifest itself in different places of the cell [6].

A covalent modification of mitochondrial proteins in consequence of the hepatotoxic action of acetaminophen was observed. Acetaminophen can connect with glutamate dehydrogenase or aldehyde dehydrogenase [6]. It has been shown that a modification of these proteins results in the impairment of their activity, leading to disorders of aldehyde to acid oxidation (detoxification). The consequence is lipid peroxidation and impaired metabolism of ammonia (involved in the synthesis of glutamine). The loss of mitochondrial balance leads to cell destruction, both by a mechanism caused by a loss of enzyme activity and by an abnormal ion flow, which reduces the ATP-ase activity, and increases the intracellular Ca^{2+} concentration in cytosol [6]. Acetaminophen's impact on the endonuclease activated by Ca^{2+} was shown. The accumulation of Ca^{2+} can cause a development of cytotoxicity and necrosis. It can lead to the DNA damage. Unrepaired DNA can contribute to the cell death [1, 4].

It has been observed that the oxidation process and Ca^{2+} imbalance can initiate an increase in the mitochondrial permeability transition (MPT), resulting in the formation of vesicles in the cell membrane and the loss of its integrity [7]. An MPT increase manifests itself by a loss of potential and by impaired oxidative phosphorylation, this also being a result of the oxidative stress. MPT causes a sudden increase in the amount of small weight soluble molecules inside the mitochondrial membrane. This leads to a strong mitochondrial dysfunction and a disorder of the energy obtaining process, which is an effect of the mitochondrial ATPase inhibition [6, 7, 11].

It is the chronic exposure rather than high doses of acetaminophen that has a toxic impact on the cell nucleus. An incorporation of acetaminophen metabolites into the DNA molecule has been shown to lead to the cell death, rapid loss of the genomic DNA integrity, DNA fragmentation, nuclear apoptosis by increasing the intensive distribution of chromatin and an almost total loss of glycogen [1]. Acetaminophen can cause unscheduled DNA synthesis in peripheral lymphocytes. In addition, one of acetaminophen metabolites – PBQ – can react with protein microtubules, inhibiting thus their formation. This in turn leads to disorders of the kinetic spindle formation in mitotic cells. Anomalies in the chromosomes arrangement are considered to be a critical event in the process of cancer cell transformation. Acetaminophen arylates the nuclear proteins and reduces the degree of chromatin binding to the inner nuclear membrane. Inhibiting the ribonucleic reductase activity, acetaminophen reduces the DNA synthesis and causes breaks in the single-stranded nuclear DNA in the liver cell. An interference of acetaminophen metabolites with the action of nucleotides involved in the cell repair mechanisms was found [1]. The non-metabolized part of acetaminophen can reduce the rate of DNA polymerization, which is essential in the reparation process. Acetaminophen was shown to modulate the signal transduction for growth factors, thereby inhibiting the cell cycle in the division phase. Consequently, acetaminophen may thus interfere with the organ regeneration, exacerbating ALF [1]. Acetaminophen has been shown to activate the c-jun (NH_2) terminal kinase (JNK), which precede the hepatocyte death. The activation of hepatic JNK may be a consequence of the oxidative stress caused by the metabolism of acetaminophen. ROS can induce and extend the JNK activation, possibly by an inactivation of cellular phosphatases. After

acetaminophen injection, the JNK stimulation in hepatocyte nuclei and in the central vein cytoplasm was observed. The highest JNK level is observed 2 hours after injection. Therefore, the JNK identification should be a potential target in the treatment of ALF induced by acetaminophen overdose [5].

After initial changes in hepatocytes, the liver cell necrosis is observed. This process spreads onto the cells embedded in the tissue. At the same time, defense mechanisms are activated. A further consequence of the changes may be their effect on blood circulation, which plays an important role in the elimination of toxic amounts of acetaminophen [1].

SUMMARY

The most frequent clinical effect of acetaminophen poisoning is liver damage. Recent studies have shown that a covalent NAPQI incorporation into the liver protein is the primary hepatotoxicity mechanism. NAPQI can cause a decrease in the levels of GSH and SH groups and an increase in the oxidative stress and lipid peroxidation, effecting the liver. Molecular changes in the cell membrane, mitochondrion, lysosome and nucleus have been found and have led to the development of acetaminophen metabolites cytotoxicity. The acetaminophen overdose can result in liver damage and tissue necrosis.

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